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CONTENTS

THE DELETERIOUS EFFECTS OF DITHIOTHREITOL (DTT) ON RED BLOOD CELL LW ANTIGENS

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Introduction

Since the first reports of the deleterious effects of 2-aminoethylisothiouronium bromide (AET)[1] and DTT[2] on antigens in the Kell blood group system, antigens in other blood group systems have been reported to be similarly affected. Antibodies directed against Yt^a, Yt^b, JMH, Yk^a, Hy, Gy^a, Kn^a, McC^a, or McC^d antigens have either weakened or no reactivity when tested against AET- or DTT-treated antigen-positive red blood cells (RBCs).[3–7] The LW^a antigen is weakened or destroyed by both DTT[8] and AET.[9] This report describes the effects of RBC DTT-treatment on the reactivity of 32 human serums and one guinea pig serum containing either anti-LW^a, -LW^b, or -LW^{ab}.

Materials and Methods

DTT-treatment of RBCs

One volume of packed RBCs was incubated with four volumes of 0.2M DTT (1,4-dithio-L-threitol, optically active, 98 percent crystalline, catalog #D9760, Sigma Co., St. Louis, MO) at 37°C for 30 min in a dry-block incubator. The treated RBCs were washed four times in isotonic saline and prepared as a 3–4 percent suspension in Alsever's solution. As a control, one volume of a second aliquot of the same RBCs was incubated with four volumes of isotonic saline and then handled in the same manner as DTT-treated RBCs.

One example each of DTT-treated or untreated R_1R_1, R_2R_2, rr, and cord reagent RBCs was tested with the serums containing anti-LW.

Serum samples

Serum from 32 patients, including Mrs. Big,[10] containing antibodies directed against LW^a, LW^b, or LW^{ab} antigens and one serum from a guinea pig injected with D-- RBCs, was tested in parallel against untreated and DTT-treated RBCs. Indirect antiglobulin reactions following incubation of test serum and cells at 37°C for 30 min in saline were graded and recorded. The serum samples were a collection obtained from various exchange programs and from patients referred between the years of 1976–1987 to the American Red Cross Reference Laboratory for identification of antibodies. All samples were stored frozen at −20°C. One example of anti-D was tested simultaneously with untreated and DTT-treated RBCs to contrast reactivity of the two specificities (anti-LW and -D).

Results

Thirty-two serums containing anti-LW reacted 1+ to 4+ with untreated R_1R_1, R_2R_2, and rr RBCs, and failed to react with the DTT-treated RBCs

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(Table 1). One serum contained anti-CDE and was tested against rr RBCs. The anti-D showed no change in reaction strength (3+) either before or after DTT-treatment of D-positive reagent RBCs. Four serums containing anti-LW^{ab} (including Mrs. Big) failed to react with DTT-treated adult RBCs but did react weakly (1+) with DTT-treated cord RBCs; untreated cord RBCs gave 4+ reactions (result not shown).

One serum was retrospectively found to contain anti-e in addition to anti-LW (not categorized). The anti-e was easily distinguishable from the anti-LW after testing the serum with DTT-treated RBCs (Table 2).

Discussion

In 1984, Konigshaus and Holland[8] reported that three serums containing anti-LW either failed to react with DTT-treated RBCs or showed a significant reduction in both titer and score as compared with untreated RBCs. Other workers have shown that the LW antigens are susceptible to pronase[11] and endo-β-*N*-acetylglucosaminidase F.[12] These enzymes are not readily available in immunohematology laboratories; however, 0.01M DTT is a reagent routinely used because it cleaves intersubunit disulfide bonds of the heavy chain of IgM antibody molecules.[13] ZZAP,[14] which is a mixture of 0.2M DTT and papain, is commonly used to treat autologous RBCs before autoadsorption of serum containing warm autoantibody; 0.2M DTT is also used to prepare reagent RBCs devoid of high-incidence antigens in a variety of blood group systems.[2,7] These cells can be used to detect additional red cell antibodies that may be masked by an antibody directed against a DTT-susceptible antigen. In addition, the DTT-treated cells can be used to distinguish anti-D from a weak anti-LW that is reacting only with D-positive RBCs.

Adsorption and elution studies were not performed with DTT-treated RBCs to determine whether this reagent completely denatures the LW antigens. Whether the antigens are completely destroyed, or whether antibodies directed against the LW antigens have a decreased affinity for DTT-treated RBCs, the treated cells can be used to advantage in identifying antibodies in the LW blood group system when rare LW(a-b-) RBCs are not available.

This study suggests that LW antigens, but not D antigens, may depend upon disulfide bonds to maintain antigen integrity. This supports previous findings.[8]

Our findings that anti-LW^{ab} reacted more strongly with cord RBCs than with adult RBCs complements previous observations on the reactions of antibodies with LW specificity.[12,15]

Table 1. Reaction strength of untreated and DTT-treated R_2R_2 RBCs after incubation with antibodies against LW antigens

		Reaction strength	
Antibody	No. examples	Untreated RBCs	DTT-treated RBCs
LW^a	10	1+ to 4+†	0
LW^b	1	2+	0
LW^{ab}	10	1+ to 4+	0
LW*	10	1+ to 4+	0
LW* and CDE	1	1+	0
LW (guinea pig)	1	1+	0
D	1	3+	3+

*Not categorized
†Indirect antiglobulin test

Table 2. Reactions of a serum containing anti-LW and anti-e

	Reaction strength	
Test RBCs	Untreated RBCs	DTT-treated RBCs
R_1R_1	4+*	3+
R_2R_2	4+	0
rr	3+	3+
Cord	4+	3+

*Indirect antiglobulin test

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UNRELIABILITY OF FIVE ELUTION TECHNIQUES IN AN ATTEMPT TO ELUTE AUTOANTI-LW AND ALLOANTI-LW

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Introduction

With the description of an antibody called Ne^a in 1981,[1,2] the current concept of LW as a three-gene system evolved. The gene *LW* is presently called LW^a, while Ne^a, now known to be the antithetical low-incidence allele of LW^a, is designated LW^b. In addition, there is an amorphic gene *lw*. The genotype *lwlw* results in the LW(a-b-) red cell phenotype. Three alloantibodies have been described: anti-LW^a, anti-LW^b, and anti-LW^{ab}. A detailed description of the LW system is given by Issitt.[3]

During the past 20 years, a number of reports have cited autoanti-LW as a cause of autoimmune hemolytic anemia.[4-7] The patients in these studies had a positive direct antiglobulin test (DAT) and sometimes had clinical signs of hemolytic anemia. In 1971, Chown et al[8] described several patients whose red blood cell (RBC) LW antigens were transiently depressed and autoanti-LW (anti-LW^a) was detected in their serum. As the LW antigen on their RBCs increased, the patients developed a positive DAT, but there was no evidence of hemolytic anemia and they were successfully transfused with LW-positive blood.

In general, Rh-positive RBCs react more strongly with LW antibodies than Rh-negative RBCs. Therefore, serologically, anti LW may mimic anti-D. Testing with cord RBCs can rule out anti-D, since anti-LW reacts equally well with cord cells of all Rh phenotypes and the reactions are usually stronger than with adult cells.[9] It has been observed that eluates prepared by various techniques from RBCs coated with autoanti-LW or alloanti-LW usually fail to yield the expected LW^a or LW^{ab} antibodies.

Materials and Methods

Two examples each of alloanti-LW^a and -LW^{ab} were tested in saline, ficin, and LISS at immediate spin (IS), 37°C and indirect antiglobulin test (IAT) against adult D-positive and D-negative RBCs of varying Rh phenotypes, D-positive and D-negative cord RBCs and LW-negative and Rh_{null} RBCs.

Adsorptions were performed using equal volumes of serum and R_1R_1 or rr untreated or enzyme-treated RBCs. The mixtures were incubated at 37°C for 1 hour. The unadsorbed and once adsorbed serums were tested by titration against R_1R_1, R_2R_2, rr, and cord (rr) red cells. The results were scored according to Marsh.[10]

Direct antiglobulin tests (DAT) were performed on the absorbing cells with both polyspecific and monospecific anti-IgG antihuman globulin serums. The DAT was read at IS and after a 5 min incubation.

Five elution techniques were tested using untreated absorbing red cells: 56°C heat, diethyl ether, digitonin acid, chloroform, and xylene.[11]

The eluates were tested against R_1R_1 and R_2R_2 adult RBCs, D-positive and D-negative cord RBCs, and LW(a-) and LW(a-b-) RBCs in LISS and with ficin-treated cells at 37°C and IAT.

Eleven examples of RBCs exhibiting a positive DAT were obtained from patients whose serums contained autoanti-LW. Eluates were prepared by one or more elution methods (ether, digitonin, chloroform).

Results

Two serums containing alloanti-LW^a and two containing alloanti-LW^{ab} reacted as expected when tested against Rh-positive and Rh-negative adult and cord RBCs. Reactivity was enhanced by ficin treatment of the RBCs. The four serums, when adsorbed with R_1R_1 or rr untreated or ficin-treated RBCs, showed some loss of titer. In no case was reactivity completely removed. Table 1 shows typical titers and scores of an alloanti-LW^a before and after adsorption with untreated R_1R_1 RBCs.

DATs performed on untreated absorbing RBCs before elutions were performed are shown in Table 2. Reactions ranged from 1–2+ with both polyspecific and monospecific antihuman globulin serums.

Table 1. Titers and scores of alloanti-LW^a before and after adsorption with R_1R_1 adult RBCs

	Titer		Score	
Cells	Unadsorbed serum	Adsorbed serum	Unadsorbed serum	Adsorbed serum
Adult R_1R_1	4	2	13	5
R_2R_2	8	4	18	10
rr	4	2	10	3
Cord rr	16	4	28	14

Table 2. Eluates prepared from untreated R_1R_1 RBCs after incubation with alloanti-LW^a or -LW^{ab}

		Elution method				
Antibody specificity	DAT*	Digitonin	56° heat	Ether	Chloroform	Xylene
LW^a	1+	–	–	–	–	–
LW^a	1+	+	–	–	–	–
LW^{ab}	2+	–	–	–	–	–
LW^{ab}	1+	–	–	–	–	–

*Direct antiglobulin test on absorbing cells